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BIOCHEMICAL SYSTEMATICS AND POPULATION GENETIC STRUCTURE OF *ANOPHELES PSEUDOPUNCTIPENNIS*, VECTOR OF MALARIA IN CENTRAL AND SOUTH AMERICA

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Abstract. An electrophoretic survey of 42 populations of *Anopheles pseudopunctipennis* collected throughout its known geographic distribution was performed to clarify the taxonomic status of this important malaria vector species. The results indicated strong differences in the allele frequencies of three enzyme loci (glycerol dehydrogenase, 6-phosphogluconate dehydrogenase, and phosphoglucomutase) of the 33 loci analyzed. No fixed electromorphic differences separate the populations of *An. pseudopunctipennis*. The populations of *An. pseudopunctipennis* showed little genetic divergence, with Nei distances ranging from 0 to 0.079. A comparison of *An. pseudopunctipennis* data with either one of three other *Anopheles* species showed a high genetic distance of 0.335 with a closely related species, *An. franciscanus*; 0.997 with *An. crucians*, and 2.355 with *An. (Nyssorhynchus) albimanus*. Geographic populations of *An. pseudopunctipennis* were classified into three clusters; one cluster included populations collected in North America (United States and Mexico) and Guatemala, one cluster included populations from Belize and South America (Colombia, Ecuador, Peru, Chile, and Argentina); and one cluster was represented by populations from the Island of Grenada (type-locality of *An. pseudopunctipennis*). Based on our isozyme analyses, we defined these clusters as three geographic populations of *An. pseudopunctipennis*. Of the two mainland populations, one extends from the southern United States south through Mexico and Guatemala, and the other extends north from southern South America through Central America to Belize. These two geographic populations converge in southern Mexico and northern Central America. One part of the convergence zone was identified in the area of eastern Guatemala and southern Belize.

Anopheles pseudopunctipennis Theobald 1901 is a major vector of human malaria in the foothills and mountainous areas of Mexico, throughout Central America, and in the Andean countries of South America. It is often the only vector present in areas at an altitude above 600 m. It is found from the United States (south of 40°N) to the northern part of Argentina (30°S) along the Andes, with an eastern extension into Venezuela and the Lesser Antilles. In the most extensive review of this species, Aitken[1] asserted that "Because of its extensive north south distribution, its inconsistencies as a vector of malaria in the Americas, and because of the conflicting reports concerning its morphology and habits, *An. pseudopunctipennis* has come to be an extremely interesting mosquito to study". Bruce-Chwatt[2] added that the extensive geographic distribution and the high level of variability of this species have led to speculation that *An. pseudopunctipennis* may represent a complex of sibling species. At the present time, the taxonomic status of *An. pseudopunctipennis* is extremely important because of its wide involvement in the transmission of human malarial pathogens.

Anopheles pseudopunctipennis was first described by Theobald[3] in 1901 from Grenada Island (Lesser Antilles). The original description was not sufficient for accurate identification of the species, resulting in confusion and misidentifications of the species.[1] Between 1907 and 1912, four different names were applied to the species (three are currently in synonymy). From 1901 to 1950, five subspecies and one variant of *An. pseudopunctipennis* were morphologically described from different areas of South America.[4] *Anopheles franciscanus* was a synonym of *An. pseudopunctipennis* for 28 years and then considered a subspecies of *An. pseudopunctipennis* for another 40 years. Only in 1972 was *An. franciscanus* elevated to the species level.[5] In 1992 and 1993, Estrada-Franco and others[6–8] stated that *An. pseudopunctipennis* constituted a complex of two species, *An. pseudopunctipennis* A, a species from central Mexico, and *An. pseudopunctipennis* B, a species from the Andes of Peru and Bolivia.

Since 1991, an extensive investigation of *An. pseudopunctipennis* from its whole geographic range has been undertaken and the results are the subject of this report. Our study of *An. pseudopunctipennis* was a genetic analysis by isozyme electrophoresis with the following emphasis: 1) analyzing genetic differentiation and variability of *An. pseudopunctipennis* within its known geographic range, from the type-locality in the Caribbean (Grenada Island) to North, Central, and South America; 2) comparing genetic differentiation and variability of *An. pseudopunctipennis* populations that correspond to the five subspecies and one variant described in the literature; 3) comparing genetic differentiation and variability of *An. pseudopunctipennis* populations from a range of altitudes; and 4) comparing the genetic profile of the *An. pseudopunctipennis* populations with other associated species of *Anopheles*.

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FIGURE 1. Collecting areas of *Anopheles pseudopunctipennis* in 10 countries throughout the species' geographic distribution. Each dot corresponds to a collection of multiple samples.

MATERIALS AND METHODS

Mosquito populations. *Anopheles pseudopunctipennis* was collected as wild larvae and pupae along its known geographic range (Figure 1) and at altitudes from sea level up to 2,500 m. The countries were chosen according to critical locations, such as the type-locality (Grenada Island), and localities where the five different subspecies and one variant of *An. pseudopunctipennis* were described,[4] as well as areas providing a spatial representation of the geographic distribution of the species. In South America, collections were made where the variant *bifoliata*[9] occurs in Colombia, where the subspecies *levicastilloi*[10] and *rivadeneirai*[10–12] occur in Ecuador, where the subspecies *neghmei* and *noei*[13] occur in Chile, and where the subspecies *patersoni*[14] occurs in Argentina. The site-specific collections were positive for all subspecies and variant except in Chile where the two subspecies of *An. pseudopunctipennis* seem to have been eradicated from the two locations described by Mann.[13] However, we found populations of *An. pseudopunctipennis* near Arica, in the northern part of Chile, approximately 200 km from the two subspecies' type-localities.

All *An. pseudopunctipennis* adults tested were collected as wild larvae or pupae and reared individually to the adult stage. Fourth instar larval and/or pupal exuviae were preserved and each specimen was recorded. An adult with associated larval and pupal skins is referred to as a taxonomic series. Each taxonomic series was identified to species and used to support the isozyme study. In most samples, an average of 40 adults (50% of both males and females) were frozen at −70°C for genetic studies and some adults were pointed on pins for taxonomic vouchers and deposited at the Smithsonian Institution (Washington, DC).

From 1991 to 1993, we collected a total of 42 samples in 10 different countries represented by more than 2,000 wild specimens of *An. pseudopunctipennis* (Table 1). To evaluate the taxonomic significance of observed differences among *An. pseudopunctipennis* populations, three other *Anopheles* species were also included in the electrophoretic data: a closely related species, *An. franciscanus* McCracken, *An. crucians* Wiedemann, and *An. (Nyssorhynchus) albimanus* Wiedemann. Wild specimens of *An. franciscanus* were collected near Redding, California, and both *An. crucians* and *An. albimanus* were collected in northern Belize. As for *An. pseudopunctipennis,* all three *Anopheles* species were collected as wild larvae and pupae and reared individually to the adult stage. An average of 40 adults per population were stored at −70°C until used in electrophoretic studies.

Electrophoresis. Isozymes were separated by horizontal starch gel electrophoresis. Staining procedures were adapted from the reports of Harris and Hopkinson,[15] Selander and others,[16] and Shaw and Prasad.[17] A total of 45 enzyme systems were screened using three different buffers: the lithium buffer system[18] (LiOH, pH 8.5), the morpholine buffer system[19] (morph, pH 6.1), and the Tris-citrate buffer system[20] (TCss, pH 6.7). Of the 45 enzyme systems tested, 25 showed good allelic resolution, including 33 putative loci (Table 2). For each locus, the most frequent electromorph was designated the 100 allele and all other alleles were measured relative to it. Additional details of the electrophoretic procedure have been described by Manguin and others,[21] and specific buffer systems and staining recipes are available upon request from one of the authors (SM).

Electromorph genotype frequencies were used as input for the computer program BIOSYS-1.[22] Analysis of each population included computation of allele frequencies, heterozygosity per locus, additional measures of genetic variability, and a test for conformance with the Hardy-Weinberg equilibrium at single loci by chi-square analysis. Differentiation among the populations was measured by F-statistics. Nei's[23] unbiased and Rogers'[24] genetic distances were clustered by the unweighted pair group method using the arithmetic average (UPGMA) to produce the phenogram.

RESULTS

A total of 42 samples, representing more than 2,000 wild specimens of *An. pseudopunctipennis*, were collected in 10 different countries of the Caribbean, North, Central, and South America (Figure 1 and Table 1). *Anopheles pseudopunctipennis* populations within each country showed negligible genetic differentiation and were combined to form a geographic area. A total of 12 geographic areas corresponding to each of the 10 countries, including northern (Monterrey) and southern (Tapachula) Mexico, and the Pacific to the Atlantic sides of Guatemala were compared in our analyses.

Heterozygosity. The isozyme comparison of 33 loci (Table 2) among the populations of *An. pseudopunctipennis* from North, Central and South America indicated that the mean heterozygosity ranged from 0.022 to 0.101, with an average (± SEM) of 0.059 (± 0.020) across all mainland populations (Table 3). Mean heterozygosity for the populations from Grenada Island was much lower[25] with a value of 0.003, which is in accordance with the usual low level of genetic variability of isolated island populations.[26]

Genetic heterogeneity. The F-statistics (F_{ST}), a measure of the amount of differentiation among subpopulations,[27] showed an average value of 0.375 and a mean index of fixation of individuals relative to the total of subpopulations

TABLE 1

Geographic information on the 42 collection sites of *Anopheles pseudopunctipennis*

Country	State	Locality and collection no.	No. of samples	No. of specimens	Elevation (m)	Longitude/latitude
United States	Texas	San Antonio: Fort Sam Houston (area 9, #1)	1	54	214	29°25′N/98°30′W
Mexico	Nuevo Leon	Monterrey: El Carmen (#1), El Rancho (#2)	2	46, 44	400	25°29-55′N/100°11-21′W
	Chiapas	Tapachula, Coatan River: El Plan (#0402.2),	1	46	480	14°47′N/92°28′W
	Chiapas	El Retiro (#0502.4), La Ceiba (#0702.1)	2	44, 40	400	15°00′N/92°28′W
	Chiapas	Zanatenco River: Tonala (#0602.1)	1	54	40	16°05′N/93°45′W
Guatemala	Escuintla	Escuintla: Guachipilin (#4), Maria Santissima (#3)	2	40, 40	250–320	14°15′N/90°47′W
	Zacapa	Usumatlan: La Palmilla (#2)	1	40	500	15°00′N/89°30′W
	El Progreso	Guastatoya: Barrial (#1), Morazan: Las Pericas (#4)	2	44, 40	600	14°50′N/90°00′W
	Baja Verapaz	San Julian: El Patal (#1)	1	5	1,400	15°15′N/90°30′W
	Alta Verapaz	Tactic (#2), Coban: El Cruce (#3)	2	40, 40	1,500	15°20′N/90°20′W
Belize	Cayo	Caves Branch (#326), Sibun River (#327-328),	2	36, 55	60	17°06-09′N/88°39-43′W
	Cayo	Rio On (#335)	1	7	480	17°59′N/88°58′W
	Stann Creek	North Stann Creek (#344)	1	40	80	17°02′N/88°32′W
Grenada	St. Patrick	Rio Sallee (#29, 31), River Sallee-Springs (#33)	2	72, 24	6	12°12′N/61°37′W
Colombia	Valle	Florida (#1–2)	1	22	1,010	3°20′N/76°12′W
Ecuador	Imbabura	Salinas (#8)	1	18	1,880	0°30′N/78°10′W
	Pichincha	Quito: Tumbaco (#7)	1	44	2,340	0°17′S/78°32′W
	Guayas	Guayaquil: Bucay (#1, 9), El Triunfo (#12)	2	29, 4	10	2°16′S/79°20-53′W
Peru	Lima	Hacienda Villa (#22, 28), Rio Chillon (#23-24), Huachipa	2	23, 44	3–100	11°50′-12°15′S/76°50′-77°00′W
	Lima	(#25-26), Cieneguilla (#27)	2	51, 36	300–320	12°00-10′S/76°50′W
	Cuzco	Quillabamba (#31)	1	2	988	12°50′S/72°50′W
Chile	Tarapaca	Arica: Rio Lluta, km25 (#1), km30 (#2), km35 (#3), km41	3	8, 8, 5	200–500	18°20′S/69°30′W
	Tarapaca	(#8-9), km53 (#10), Rio Azapa (#4)	3	44, 30, 13	270–850	18°20-30′S/69°30-70°00′W
Argentina	Salta	Puente Polares (#12), Alemania (#13), Santa Barbara (#14)	3	5, 16, 26	1,160–1,440	25°00-50′S/65°15′W
	Tucuman	Rio Tapia (#16), Rio Vipos (#17)	2	22, 27	700–800	26°30-40′S/65°20′W

TABLE 2

Electrophoretically detected enzyme systems of *Anopheles pseudopunctipennis*

Enzyme system	E.C. number*	Symbol	No. of loci†	Buffer‡
Aconitase	4.2.1.3	ACON	2	TCss
Adenylate kinase	2.7.4.3	AK	1	TCss
Aldehyde oxidase	1.2.3.1	AO	1	LiOH
Arginine kinase	2.7.3.3	ARGK	1	LiOH
Esterase	3.1.1.1	EST	1	Morph
Fumarase	4.2.1.2	FUM	1	TCss
Glutamate oxaloacetate transaminase	2.6.1.1	GOT	2	Morph
Glutathione reductase	1.6.4.2	GR	2	TCss
Glycerol dehydrogenase	1.1.1.72	GCD	1	Morph
α-glycerophosphate dehydrogenase	1.1.1.8	GPDH	1	Morph
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	G3PDH	1	Morph
Hexokinase	2.7.1.1	HK	2	Morph
β-hydroxyacid dehydrogenase	1.1.1.30	HAD	1	Morph
Isocitrate dehydrogenase	1.1.1.42	IDH	2	Morph
Lactate dehydrogenase	1.1.1.27	LDH	1	LiOH
Leucine amino peptidase	3.4.11.1	LAP	2	LiOH
Malate dehydrogenase	1.1.1.37	MDH	1	Morph
Malic enzyme	1.1.1.40	ME	1	Morph
Mannose-6-phosphate isomerase	5.3.1.8	MPI	1	Morph
6-phosphogluconate dehydrogenase	1.1.1.44	6PGD	1	Morph
Phosphoglucomutase	5.4.2.2	PGM	1	Morph
Phosphoglucose isomerase	5.3.1.9	PGI	1	LiOH
Pyruvate kinase	2.7.1.40	PK	2	TCss
Sorbitol dehydrogenase	1.1.1.14	SDH	1	LiOH
Triose phosphate isomerase	5.3.1.1	TPI	2	Morph

* Enzyme commission (E.C.) number.
† Number of scorable bands per phenotype.
‡ Refers to the electrophoresis buffer (see Materials and Methods).

TABLE 3

Measures of genetic variation of *Anopheles pseudopunctipennis* (1–12) and *An. franciscanus* (13), *An. crucians* (14), and *An. albimanus* (15)*

Populations	No. of samples	Mean sample size/locus	Mean no. of alleles/locus	% polymorphic loci†	Mean heterozygosity	
					Direct count	Hardy-Weinberg equilibrium (expected)‡
1. United States	1	52.2	1.6	48.5	0.101	0.098
		(0.9)	(0.1)		(0.028)	(0.027)
2. Monterrey, Mexico	2	75.0	2.2	72.7	0.066	0.078
		(4.9)	(0.2)		(0.017)	(0.020)
3. Tapachula, Mexico	4	147.7	2.5	78.8	0.061	0.065
		(8.6)	(0.2)		(0.016)	(0.017)
4. Pacific, Guatemala	4	67.5	2.0	60.6	0.044	0.050
		(3.9)	(0.2)		(0.012)	(0.014)
5. Atlantic, Guatemala	4	191.2	2.4	72.7	0.050	0.052
		(5.4)	(0.2)		(0.013)	(0.014)
6. Belize	4	105.7	1.8	66.7	0.074	0.069
		(6.8)	(0.1)		(0.032)	(0.025)
7. Grenada	2	69.5	1.1	12.1	0.003	0.003
		(3.6)	(0.1)		(0.002)	(0.002)
8. Colombia	1	20.3	1.5	45.5	0.059	0.067
		(0.6)	(0.1)		(0.017)	(0.021)
9. Ecuador	4	87.7	1.7	51.5	0.069	0.072
		(3.6)	(0.1)		(0.023)	(0.024)
10. Peru	5	125.8	1.9	57.6	0.039	0.043
		(7.3)	(0.2)		(0.011)	(0.013)
11. Chile	6	96.6	1.5	39.4	0.022	0.021
		(4.6)	(0.1)		(0.008)	(0.008)
12. Argentina	5	80.1	1.8	51.5	0.061	0.065
		(4.9)	(0.2)		(0.021)	(0.023)
13. *An. franciscanus*	1	9.5	1.4	33.3	0.084	0.088
		(0.3)	(0.1)		(0.027)	(0.028)
14. *An. crucians*	1	32.2	1.5	39.4	0.078	0.071
		(2.2)	(0.1)		(0.029)	(0.025)
15. *An. albimanus*	1	35.8	1.6	30.3	0.051	0.057
		(2.5)	(0.2)		(0.023)	(0.026)

* Values in parentheses are standard errors.
† A locus is considered polymorphic if the frequency of the most common allele does not exceed 0.99.
‡ Unbiased estimate.

TABLE 4

F-statistics analysis of polymorphic loci in 42 populations of *Anopheles pseudopunctipennis**

Locus	F_{IS}	F_{IT}	F_{ST}
Acon-1	0.020	0.047	0.027
Acon-2	−0.006	−0.002	0.004
Ak-3	0.016	0.056	0.041
Ao	−0.019	−0.002	0.017
Argk	0.330	0.348	0.027
Est	0.140	0.363	0.259
Fum	0.075	0.089	0.015
Gcd	0.084	0.776	0.755
Got-1	−0.031	−0.007	0.023
Got-2	0.017	0.152	0.138
Gpdh	−0.071	−0.010	0.057
G3pdh	−0.000	0.019	0.019
Gr	0.051	0.231	0.189
Had	0.008	0.053	0.045
Hk-1	0.212	0.390	0.226
Hk-2	0.015	0.032	0.017
Idh-1	−0.383	−0.057	0.235
Idh-2	0.072	0.146	0.079
Lap-1	0.082	0.133	0.055
Lap-2	0.037	0.068	0.032
Ldh	−0.151	−0.019	0.115
Mdh	−0.009	−0.003	0.006
Me	0.169	0.176	0.009
Mpi	0.205	0.255	0.063
6Pgd	0.100	0.618	0.576
Pgi	−0.004	−0.001	0.003
Pgm	0.069	0.635	0.608
Pk-1	−0.016	−0.006	0.011
Pk-2	−0.050	0.027	0.073
Sdh	−0.053	0.111	0.156
Tpi-1	−0.075	−0.007	0.063
Tpi-2	0.178	0.204	0.031
Mean	0.036	0.397	0.375

*F_{IS} = fixation indices of individuals relative to the total subpopulations; F_{IT} = fixation indices of individuals relative to the total populations; F_{ST} = F statistics. For definitions of loci, see Table 2.

(F_{IS}) value of 0.036 when all *An. pseudopunctipennis* populations were analyzed (Table 4). Three loci, glycerol dehydrogenase (*Gcd*), 6-phosphogluconate dehydrogenase (*6Pgd*), and phosphoglucomutase (*Pgm*), among a total of 33 showed great differentiation, with values of 0.755, 0.576, and 0.608, respectively. In the case of *Gcd* (Figure 2A), the populations of *An. pseudopunctipennis* from North America (United States and Mexico) and Guatemala have a high frequency (92%) for allele Gcd_{122} (Table 5), whereas all the other *An. pseudopunctipennis* populations showed a high frequency (90–100%) for allele Gcd_{100}. For *6Pgd* (Figure 2B), all *An. pseudopunctipennis* populations have a high frequency (84–98%) for allele $6Pgd_{100}$, except the ones from Grenada, which have a very high frequency (100%) for allele $6Pgd_{128}$. With *Pgm* (Figure 2C), North America, Guatemala, and Grenada have a high frequency (90–100%) for allele Pgm_{100}, whereas all the *An. pseudopunctipennis* populations from South America and Belize have a high frequency (87%) for allele Pgm_{123}. No fixed differences for the three loci (*Gcd, 6Pgd,* and *Pgm*) or any other loci have been found for all *An. pseudopunctipennis* populations (Table 5).

Electromorph frequency data for the 33 enzyme loci studied with all *An. pseudopunctipennis* populations are shown in Appendix A. Only one locus, glutathione reductase-2, was monomorphic in all different populations and species. No electrophoretic activity was shown by *An. crucians* for isocitrate dehydrogenase-2 and by *An. albimanus* for leucine amino peptidase-2, mannose-6-phosphate isomerase (*Mpi*), and sorbitol dehydrogenase. Significant departures from Hardy-Weinberg expectations were observed in only 10 cases (loci indicated by ‡ in Appendix A) among the 396 comparisons (2.5%). The resolution of esterase and *Mpi* was sometimes poor, which might have introduced some scoring errors. Five populations demonstrated a deficiency of heterozygotes from expected proportions in either arginine kinase, fumarase, *Gcd,* glyceraldehyde-3-phosphate dehydrogenase, malic enzyme, *Mpi,* or *Pgm.* Since rare alleles were involved, little meaning can be attached to these significant (probability ≤ 1%) deviations.

Genetic structure of subpopulations. From the frequency data, both Nei's[13] unbiased and Rogers'[14] distance matrices were calculated for the different populations and species (Table 6). Nei's[13] unbiased distance was chosen for ease in comparing these results with those of Estrada-Franco and others[7] and were clustered by UPGMA to produce the phenograms shown in Figures 3 and 4. Phenograms produced using other distance measures, such as Nei's,[28] Rogers,[24] modified Rogers,[27] Cavalli-Sforza and Edwards chord and arc,[29] produced nearly identical branching patterns. The three major groupings shown on Figure 3 were produced by each of the methods listed above.

Populations of three *An. pseudopunctipennis* subspecies, *levicastilloi* and *rivadeneirai* from Ecuador, and *patersoni* from Argentina, and the variant *bifoliata* from Colombia, were electrophoretically compared with the other populations from South America. The results showed no significant differences among the populations.

The comparison of all populations of *An. pseudopunctipennis* showed some differences in the allele frequencies, but the Nei's index of genetic distance indicated a high degree of similarity, with values ranging from 0 to 0.079 (Table 6). The phenogram showed three clusters of *An. pseudopunctipennis* populations (Figure 3): one cluster from North America (United States and Mexico) and Guatemala, a second cluster from Belize and South America (Colombia, Ecuador, Chile, Peru, and Argentina), and a third cluster represented by populations from Grenada only. The phenogram indicated that *An. pseudopunctipennis* populations from North America and Guatemala formed a group with very low genetic distance (less than 0.010). The F_{ST} analysis using Wright's categories[27] of the subpopulations represented by the North America-Guatemala group indicated a negligible differentiation, with a mean value of 0.049. The second group included populations from Belize and South America with genetic distances less than 0.024. The F_{ST} analysis for populations from South America and from Belize-South America showed moderate differentiation, with mean values of 0.186 and 0.192, respectively. The third group, represented by populations from Grenada, had a genetic distance at a level of 0.060 if compared with populations from North, Central, and South America. The two major differences between the populations of Grenada and all the others were 1) the different allele frequencies for *6Pgd* and 2) the lack of heterozygotes due to isolation of the Grenada populations.

Genetic structure compared with other *Anopheles* species. The comparison of *An. pseudopunctipennis* populations

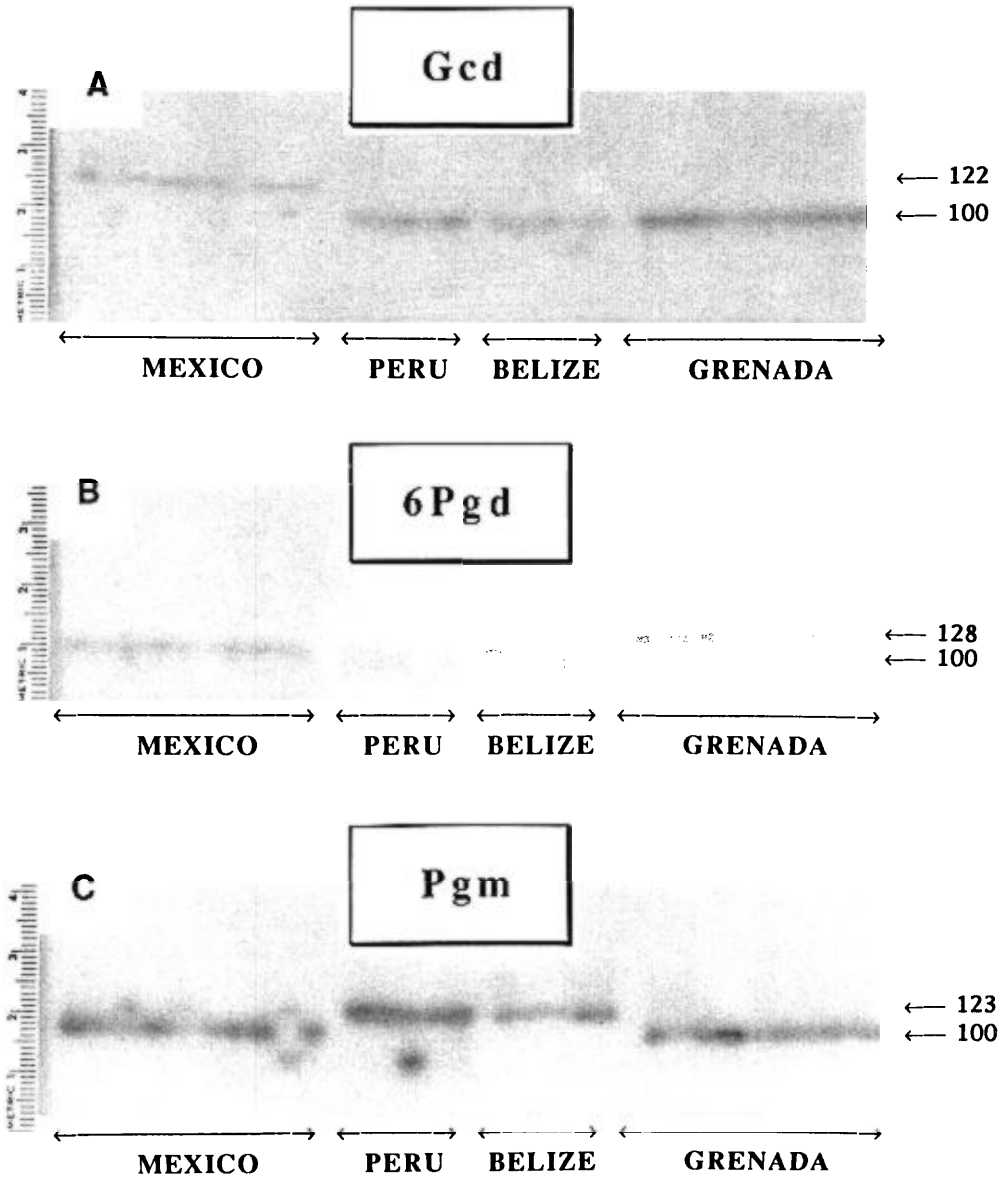

FIGURE 2. Electrophoretic pattern of three enzyme loci in *Anopheles pseudopunctipennis* populations from Mexico, Peru, Belize, and Grenada. **A,** *Gcd* = glycerol dehydrogenase. **B,** *6Pgd* = 6-phosphogluconate dehydrogenase. **C,** *Pgm* = phosphoglucomutase. Values on the right are the relative mobilities of alleles.

with either of the three *Anopheles* species, *An. franciscanus, An. crucians,* and *An. albimanus,* showed increasingly higher genetic distances (Figure 4). When the comparison involves two species of the subgenus *Anopheles,* the genetic distance varies from 0.335 with *An. franciscanus,* a closely related species of *An. pseudopunctipennis,* to 0.997 with *An. crucians.* In the case of the comparison with *An. albimanus,* which belongs to the *Nyssorhynchus* subgenus, the genetic distance is much higher, with a value of 2.355.

These comparisons emphasize the great similarity existing among the populations of *An. pseudopunctipennis* from all geographic areas.

DISCUSSION

Results of this study on *An. pseudopunctipennis* indicate that strong allele frequency differences occurred for three of the 33 loci (*Gcd, 6Pgd,* and *Pgm*), but no fixed differences were found for any of these loci. The mean heterozygosity, which reflects the genetic variability, was very low for *An. pseudopunctipennis* populations on Grenada Island with a value of 0.003 (Table 3). In contrast, the mean heterozygosity of mainland *An. pseudopunctipennis* populations varied from 0.022 to 0.101, with an average (± SEM) of 0.059 (± 0.020). The mean ± SEM heterozygosity for *An. pseudopunctipennis* seemed to be lower than that expected (0.115 ± 0.009) for other Diptera groups.[30]

In the case of *An. pseudopunctipennis* populations from Grenada and Chile, the lack of alleles for *6Pgd* is part of the general phenomenon of low allelic polymorphisms found in these two populations (Table 3). These findings are compatible with the general observation that species or populations that are distributed over a variety of environmental

TABLE 5

Relative allele frequencies for three loci of *Anopheles pseudopunctipennis* clustered in three geographic groups and *An. franciscanus, An. crucians,* and *An. albimanus**

		An. pseudopunctipennis			*An. franciscanus*	*An. crucians*	*An. albimanus*
Locus†	Allele‡	United States, Mexico, Guatemala	Belize, South America	Grenada	United States	Belize	Belize
Gcd	n	271	299	32	10	4	3
	250	0	0	0	0	0	**1.000**
	173	0.004	0	0	0	**1.000**	0
	142	0.020	0	0	0	0	0
	122	**0.917**	0.003	0	0	0	0
	100	0.057	**0.901**	**1.000**	**0.950**	0	0
	77	0.002	0.095	0	0	0	0
	53	0	0	0	0.050	0	0
	(H)	0.148	0.144	0	0.100	0	0
6Pgd	n	617	618	95	10	39	43
	225	0	0	0	0	0	0.012
	191	0	0	0	0	0	**0.965**
	147	0.008	0.014	0	0	0	0.023
	128	0.002	0.014	**1.000**	0	0	0
	100	**0.981**	**0.839**	0	**1.000**	**0.974**	0
	54	0.006	0.121	0	0	0.026	0
	23	0.004	0.013	0	0	0	0
	(H)	0.039	0.194	0	0	0.051	0.070
Pgm	n	616	609	95	10	40	42
	151	0.006	0.002	0	0	0	**0.976**
	123	0.075	**0.865**	0	0	0	0.012
	100	**0.899**	0.126	**1.000**	**0.900**	**0.975**	0.012
	75	0.020	0.006	0	0.100	0.025	0
	(H)	0.172	0.163	0	0.200	0.050	0.048

*Bold numbers indicate the highest frequency of each locus and each population.
†*Gcd* = glycerol dehydrogenase; *6Pgd* = 6-phosphogluconate dehydrogenase; *Pgm* = phosphoglucomutase.
‡n = no. of specimens; H = heterozygosity (direct count) per locus.

conditions are most likely to be genetically heterozygous or polymorphic. Conversely, species or populations limited in distribution or restricted to special habitats are less polymorphic.[26] The Grenada Island populations differed from mainland populations by having allele frequency differences for *6Pgd* and by lacking heterozygotes due to geographic isolation. The Grenada Island populations showed a low allelic polymorphism of 12.1% compared with the mainland populations, with values ranging from 39.4% to 78.8%. Average heterozygosity over the 33 loci examined was also low (3%), reflecting founder effects.[31] The relatively higher Nei genetic distance obtained for the Grenada populations (0.079) compared with mainland populations (Table 6) is partly due to differences in allelic polymorphism. In particular, the absence of rare alleles is an indication that a major reduction in the gene pool occurred in the recent evolutionary history of this island population.[32] This phenomenon can result from a drastic reduction in population size followed by population expansion from a small number of individuals. Such fluctuations in population size are common occurrences in insect colonies.[32] The significant loss of alleles and geographic isolation of *An. pseudopunctipennis* populations of Grenada are limiting factors for gene flow between populations of the island and the mainland. As a result, *An. pseudopunctipennis* populations of Grenada may eventually form a distinct species through allopatric speciation.[33] However,

TABLE 6

Matrix of genetic distance of *Anopheles pseudopunctipennis**

Population	1	2	3	4	5	6	7	8	9	10	11	12
1. United States	–	0.051	0.047	0.051	0.050	0.127	0.121	0.109	0.102	0.113	0.112	0.124
2. Monterrey, Mexico	0.008	–	0.028	0.027	0.032	0.098	0.090	0.089	0.077	0.083	0.082	0.102
3. Tapachula, Mexico	0.008	0.001	–	0.017	0.017	0.094	0.089	0.097	0.078	0.087	0.081	0.105
4. Pacific, Guatemala	0.009	0.001	0.000	–	0.014	0.091	0.082	0.098	0.077	0.085	0.077	0.103
5. Atlantic, Guatemala	0.009	0.002	0.000	0.000	–	0.092	0.082	0.095	0.074	0.084	0.081	0.102
6. Belize	0.067	0.049	0.053	0.054	0.056	–	0.093	0.075	0.067	0.051	0.041	0.069
7. Grenada	0.079	0.057	0.062	0.061	0.063	0.064	–	0.087	0.078	0.073	0.070	0.076
8. Colombia	0.052	0.040	0.049	0.055	0.054	0.027	0.059	–	0.062	0.055	0.058	0.066
9. Ecuador	0.045	0.029	0.034	0.035	0.036	0.017	0.046	0.022	–	0.054	0.053	0.068
10. Peru	0.067	0.049	0.055	0.058	0.060	0.010	0.057	0.020	0.014	–	0.025	0.030
11. Chile	0.067	0.048	0.054	0.056	0.059	0.009	0.062	0.021	0.014	0.002	–	0.042
12. Argentina	0.076	0.059	0.067	0.069	0.072	0.018	0.052	0.026	0.021	0.004	0.011	–

*Values above diagonal are Rogers[24] genetic distances; values below diagonal are Nei[23] unbiased (1978) genetic distances.

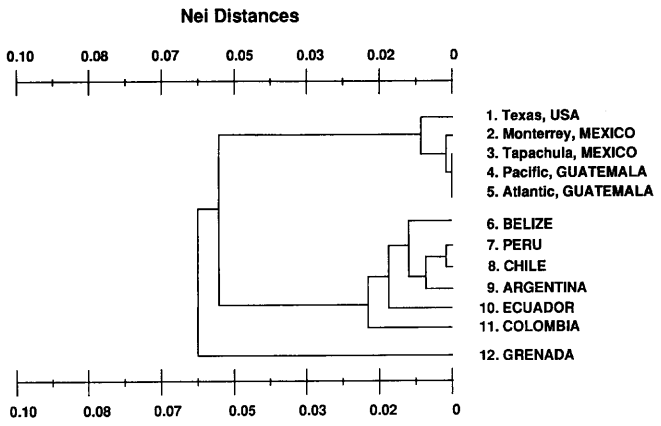

FIGURE 3. Unweighted pair group method using the arithmetic average phenogram from Nei's[13] unbiased genetic distance matrix for all *Anopheles pseudopunctipennis* populations (cophenetic correlation = 0.936).

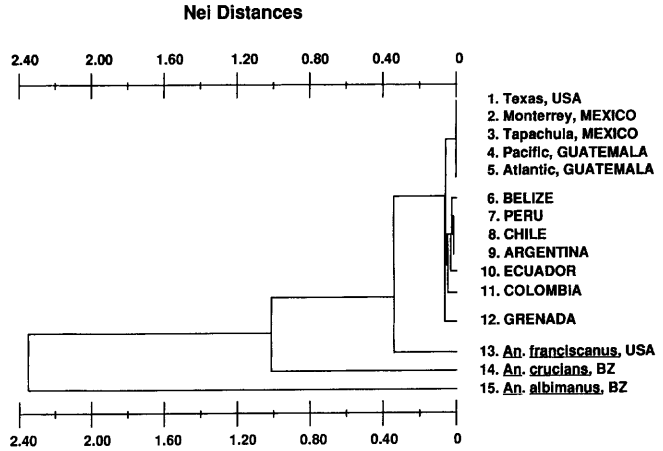

FIGURE 4. Unweighted pair group method using the arithmetic average phenogram from Nei's[13] unbiased genetic distance matrix for all *Anopheles pseudopunctipennis* populations and *An. franciscanus, An. crucians,* and *An. albimanus* (cophenetic correlation = 0.990).

the decreasing presence of *An. pseudopunctipennis* on the island of Grenada, possibly due to reduced genetic variability accompanied by a diminished capacity of the species to adapt to environmental changes,[25] might result in the species disappearing from Grenada.

The collection site in northern Chile also represented a restricted ecosystem. The Tarapaca region where *An. pseudopunctipennis* was collected in Chile is bordered by the Pacific Ocean to the west, the Andes to the east, and deserts to the north and south. *Anopheles pseudopunctipennis* was collected along two rivers flowing from the Andes, the Rio Azapa and the Rio Lluta, and was much more abundant along the latter river. Because of geographic barriers, *An. pseudopunctipennis* populations in Chile had more restricted distributions and possibly more reduced gene flow than any other populations on the continent. In such a restricted environment, the bottleneck effect is expected to produce populations less polymorphic.

Insect colonies are characterized by rapid genetic changes, such as loss of heterozygosity.[32] Loss of population heterozygosity in colonies probably reflects what occurs in restricted and isolated environments with the gradual loss of polymorphic alleles. Although rare alleles contribute little to a natural population's level of heterozygosity, these are the very alleles that are likely to be missing from a colony.[34] The decrease or absence of rare alleles is a small but sensitive indicator of the more general phenomenon of loss in genetic heterogeneity, particularly in species with high genetic variability.[32]

Overall genetic distance within *An. pseudopunctipennis* populations was low, with values ranging from 0 to 0.079. These genetic distances are markedly lower than the value of 0.16 suggested by Avise[35] as the lower limit for conspecific populations. Values of F_{ST} (Table 4) show great differentiation among *An. pseudopunctipennis* populations. This level of differentiation is due to low heterozygosities of populations from two specific countries, Grenada and Chile; differences in the allele frequency of three loci among 33; and the broad geographic spread of sample sites. Regardless, the moderate mean of F_{IS} with a value of 0.036 suggests that random mating among the populations of *An. pseudopunctipennis* is occurring[27] (Table 4).

As clearly indicated in the phenogram (Figure 3), *An. pseudopunctipennis* populations are clustered into three genetically associated groups: 1) North America and Guatemala, 2) Belize and South America, and 3) Grenada Island. A portion of the convergence zone or suture zone[36] where geographic populations 1 and 2 meet is located in the 300-km area around the southern border between Guatemala and Belize (Figure 5). Where the convergence zone is located outside of this area is not known. The interface zone between the two populations may be static and define where the North and South American populations came together after a long period of isolation. Alternatively, the convergence zone may represent only the most recent location of a mobile border between two merging geographic populations.

Our findings have some similarities with the results of Estrada-Franco and others,[7] who found "clear distinctions between populations from South America and Mexico at two loci, *Gcd* and *Pgm*". However, unlike their statement that "the major contribution to genetic divergence is the result of fixed differences in the *Gcd* and *Pgm* loci between Mexico and South America," we found no fixed differences either at these or other loci. Comparisons of Nei's genetic distances show that between populations of *An. pseudopunctipennis* and *An. franciscanus,* values are at the level of 0.335, and among *An. pseudopunctipennis* populations, values are less than 0.08. The differences in the genetic distances between *An. pseudopunctipennis* and *An. franciscanus* populations confirms the separation of the two closely related species and reinforces our finding of genetic homogeneity among *An. pseudopunctipennis* populations.

Our study represents an advance over earlier work on population genetics of *An. pseudopunctipennis.*[6-8] We used 25 enzyme systems for which were scored 33 putative loci that were used to compare *An. pseudopunctipennis* populations. A large number of sites were sampled, providing 42 samples spread from the northern to southern limits of the species'

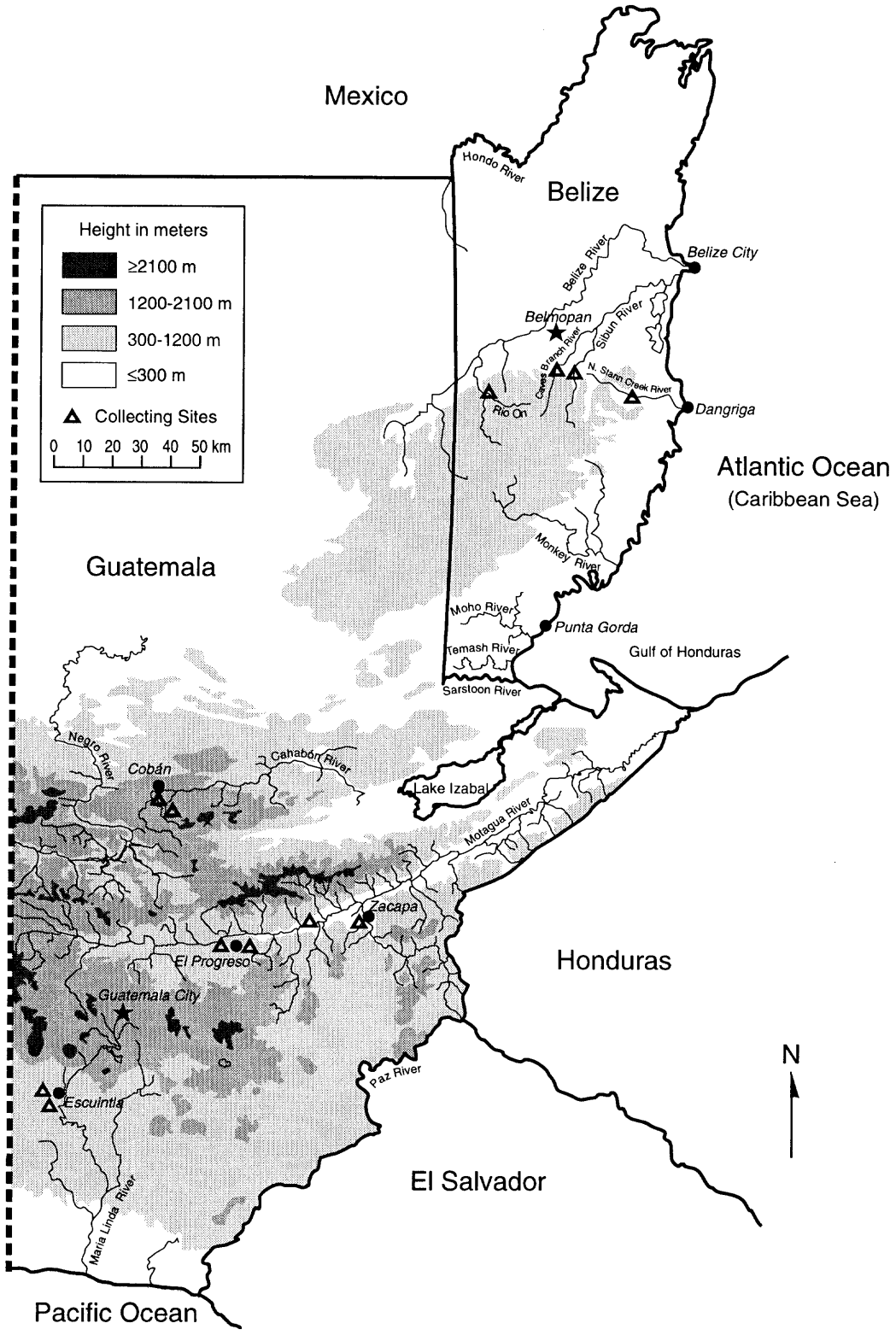

FIGURE 5. Convergence zone between *Anopheles pseudopunctipennis* populations from Guatemala and Belize.

distribution, with an eastern extension in the Caribbean. Samples were obtained from one Caribbean, two North American, two Central American, and five South American countries. Populations from Grenada, the type-locality of *An. pseudopunctipennis* species, were compared with mainland populations. This was an important part of the study since specimens from Grenada represent the true *An. pseudopunctipennis.* Additionally, heterozygosity levels were measured for *An. pseudopunctipennis* populations as well as for three other *Anopheles* species.

In conclusion, our study showed distinct differences in the allele frequencies of *Gcd* and *Pgm* between North and South America, although we distinguished overlapping frequencies. A comparison of *An. pseudopunctipennis* populations with three other *Anopheles* species, *An. franciscanus, An. crucians* (both of the subgenus *Anopheles*), and *An. albimanus* (subgenus *Nyssorhynchus*), provided a perspective for interpretation of Nei's genetic distances.

Based on the evidence of our isozyme analyses, the 42 samples of *An. pseudopunctipennis* were clustered into three geographic populations represented by 1) North America (United States and Mexico) and Guatemala, 2) Belize and South America (Colombia, Ecuador, Peru, Chile, and Argentina), and 3) Grenada Island. Of the two mainland populations (1 and 2), one extends from the southern United States through Mexico and Guatemala, and the other extends north from southern South America through Central America to Belize. These two geographic populations converge in southern Mexico and northern Central America. Part of a convergence zone, situated at the vicinity of the southern border between Belize and Guatemala, was defined for the two mainland geographic populations of *An. pseudopunctipennis.*

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APPENDIX A

Electromorph frequency data for the 33 enzyme loci studied for all *Anopheles pseudopunctipennis* populations studied and for *An. franciscanus, An. crucians,* and *An. albimanus*

		Population														
Locus*	Allele†	United States Texas	Mexico Monterrey	Mexico Tapachula	Guatemala Pacific	Guatemala Atlantic	Belize	Grenada	Colombia	Ecuador	Peru	Chile	Argentina	*An. franciscanus*	*An. crucians*	*An. albimanus*
Acon-1	n	32	75	131	28	121	96	60	4	18	113	90	69	4	28	37
	116	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.014
	108	0	0.007	0.042	0.018	0.025	0	0.008	0	0	0.009	0	0.036	0	0	0.959
	100	1.000	0.973	0.912	0.982	0.921	0.979	0.992	1.000	1.000	0.973	1.000	0.964	1.000	1.000	0.027
	91	0	0.020	0.046	0	0.054	0.021	0	0	0	0.018	0	0	0	0	0
	(H)	0	0.053	0.153	0.036	0.140	0.042	0.017	0	0	0.053	0	0.072	0	0	0.081
Acon-2	n	54	90	172	80	209	121	79	22	95	145	108	96	10	32	42
	−85	0	0.006	0	0	0.002	0.008	0	0	0	0.003	0	0	0	0.016	1.000
	−100	1.000	0.994	1.000	1.000	0.998	0.992	1.000	1.000	1.000	0.997	1.000	1.000	1.000	0.984	0
	(H)	0	0.011	0	0	0.005	0.017	0	0	0	0.007	0	0	0	0.031	0
Ak-3	n	54	87	178	80	209	130	85	21	95	151	116	95	10	40	35
	129	0.019	0.011	0.003	0	0.007	0.008	0	0.071	0.005	0.063	0.009	0.074	0	1.000	1.000
	100	0.981	0.937	0.997	0.994	0.990	0.992	1.000	0.905	0.984	0.927	0.991	0.868	0.950	0	0
	73	0	0.052	0	0.006	0.002	0	0	0.024	0.011	0.010	0	0.053	0	0	0
	40	0	0	0	0	0	0	0	0	0	0	0	0.005	0.050	0	0
	(H)	0.037	0.103	0.006	0.013	0.019	0.015	0	0.190	0.032	0.119	0.017	0.242	0.100	0	0
Ao	n	54	90	172	80	209	127	79	22	95	145	108	95	10	40	43
	100	0.981	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	95	0.019	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	(H)	0.037	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Argk	n	54	90‡	168	80	209	113	79	22	95	145	108	96	10	40	43
	112	0	0.011	0	0	0	0	0	0	0	0	0	0	0	1.000	1.000
	105	1.000	0.989	0.994	1.000	1.000	0.960	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0	0
	94	0	0	0.006	0	0	0.040	0	0	0	0	0	0	0	0	0
	(H)	0	0	0.012	0	0	0.062	0	0	0	0	0	0	0	0	0
Est	n	53	87‡	58	62‡	185	88	47	22	94	141	86	95	10	18	40
	112	0	0	0.009	0	0	0	0	0	0	0	0	0	0	0	0
	105	0	0.017	0.043	0.008	0.019	0.051	0	0	0.106	0.021	0	0	0	0	0.988
	100	0.594	0.747	0.802	0.919	0.865	0.926	1.000	0.25	0.856	0.918	0.948	0.916	0.150	0	0.013
	95	0.368	0.224	0.147	0.073	0.108	0.023	0	0.75	0.037	0.060	0.047	0.084	0.750	0	0
	91	0.038	0.011	0	0	0.008	0	0	0	0	0	0.006	0	0.100	0.917	0
	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0.083	0
	(H)	0.585	0.218	0.328	0.129	0.205	0.125	0	0.227	0.223	0.135	0.105	0.126	0.400	0.167	0.025
Fum	n	54	90‡	171	80	209	127	79	22	95	145	108	96	10	40	43
	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.012
	168	0	0.028	0.003	0	0.005	0.008	0	0	0.005	0	0	0	0	0	0.988
	100	1.000	0.950	0.994	0.988	0.988	0.992	1.000	1.000	0.995	0.986	0.991	1.000	1.000	1.000	0
	35	0	0.022	0.003	0.013	0.007	0	0	0	0	0.014	0.009	0	0	0	0
	(H)	0	0.078	0.012	0.025	0.024	0.016	0	0	0.011	0.028	0.019	0	0	0	0.023
Gcd	n	52	14	28	24‡	153	31	32	21	95	73	58	21	10	4	3
	250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.000
	173	0	0	0	0.021	0.003	0	0	0	0	0	0	0	0	1.000	0
	142	0.010	0	0.018	0.021	0.026	0	0	0	0	0	0	0	0	0	0
	122	0.962	0.821	0.893	0.896	0.918	0	0	0.024	0	0.007	0	0	0	0	0
	100	0.029	0.179	0.089	0.042	0.052	0.919	1.000	0.976	0.726	0.993	1.000	1.000	0.950	0	0
	77	0	0	0	0.021	0	0.081	0	0	0.274	0	0	0	0	0	0
	53	0	0	0	0	0	0	0	0	0	0	0	0	0.050	0	0
	(H)	0.077	0.214	0.214	0.125	0.157	0.161	0	0.048	0.379	0.014	0	0	0.100	0	0

APPENDIX A
Continued

Locus*	Allele†	Population														
		United States Texas	Mexico Monterrey	Mexico Tapachula	Guatemala Pacific	Guatemala Atlantic	Belize	Grenada	Colombia	Ecuador	Peru	Chile	Argentina	*An. franciscanus*	*An. crucians*	*An. albimanus*
Got-1	n	54	10	120	36	157	25	22	21	95	32	44	21	10	4	3
	112	0	0	0	0	0.003	0	0	0	0.042	0.016	0	0	0	0	0
	100	1.000	1.000	0.988	1.000	0.978	1.000	1.000	1.000	0.947	0.969	1.000	1.000	0.050	0	0
	91	0	0	0.008	0	0.006	0	0	0	0.011	0.016	0	0	0.850	1.000	0
	78	0	0	0.004	0	0.013	0	0	0	0	0	0	0	0.100	0	1.000
	(H)	0	0	0.025	0	0.045	0	0	0	0.105	0.063	0	0	0.300	0	0
Got-2	n	54	10	123	42	160	43	50	21	95	32	44	21	10	12	3
	80	0	0	0	0	0	0	0	0.048	0	0	0	0	0	0	0
	−37	0.324	0.200	0.297	0.250	0.253	0.081	0	0	0	0	0.023	0	1.000	0	0
	−100	0.667	0.800	0.703	0.750	0.741	0.919	1.000	0.952	1.000	0.906	0.977	1.000	0	1.000	1.000
	−163	0.009	0	0	0	0.006	0	0	0	0	0.094	0	0	0	0	0
	(H)	0.537	0.400	0.333	0.310	0.356	0.163	0	0.095	0	0.125	0.045	0	0	0	0
Gpdh	n	54	89	172	80	209	127	80	21	95	145	108	96	10	39	43
	178	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.023
	137	0.093	0.011	0.003	0.006	0.005	0	0	0	0	0	0	0	0	0.026	0.965
	100	0.907	0.989	0.985	0.994	0.988	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.962	0.012
	60	0	0	0.012	0	0.007	0	0	0	0	0	0	0	0	0	0
	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0.013	0
	(H)	0.185	0.022	0.029	0.013	0.024	0	0	0	0	0	0	0	0	0.077	0.070
G3pdh	n	54	90	172	80	208‡	127	80	21	95	145	108	96	10	40	43
	134	0	0	0.003	0	0.017	0.016	0.031	0.048	0.026	0	0.005	0	0	0	0
	100	1.000	1.000	0.997	1.000	0.971	0.984	0.969	0.952	0.974	1.000	0.995	1.000	0	1.000	0
	70	0	0	0	0	0.012	0	0	0	0	0	0	0	0	0	1.000
	32	0	0	0	0	0	0	0	0	0	0	0	0	1.000	0	0
	(H)	0	0	0.006	0	0.048	0.031	0.063	0.095	0.053	0	0.009	0	0	0	0
Gr-1	n	40	89	172	80	205	127	73	18	95	144	108	95	8	34	40
	118	0	0.017	0	0	0	0	0	0	0	0	0	0	0	0.706	0.587
	109	0.412	0.011	0.003	0.019	0.012	0.016	0	0.056	0.042	0.069	0	0.153	0.562	0.294	0.400
	100	0.587	0.966	0.994	0.981	0.971	0.984	1.000	0.944	0.958	0.927	1.000	0.821	0.438	0	0.013
	92	0	0.006	0.003	0	0.017	0	0	0	0	0.003	0	0.026	0	0	0
	(H)	0.425	0.067	0.012	0.038	0.059	0.031	0	0.111	0.084	0.132	0	0.284	0.625	0.412	0.350
Gr-2	n	40	90	172	80	205	127	73	18	95	145	108	95	8	40	43
	155	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.000
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0
	(H)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Had	n	54	89	172	80	209	127	80	22	95	145	108	95	10	40	43
	132	0	0	0	0	0	0	0	0	0	0	0	0	0.050	0	0
	119	0	0.006	0	0	0.002	0	0	0	0	0.003	0	0.016	0	0	0.512
	100	0.870	0.893	0.916	0.919	0.976	0.996	0.994	1.000	1.000	0.979	0.954	0.979	0.900	0	0.477
	77	0.130	0.096	0.070	0.075	0.017	0.004	0.006	0	0	0	0.046	0.005	0.050	0	0.012
	50	0	0.006	0.015	0.006	0.005	0	0	0	0	0.010	0	0	0	0.025	0
	41	0	0	0	0	0	0	0	0	0	0.007	0	0	0	0	0
	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0.962	0
	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0.013	0
	(H)	0.259	0.157	0.157	0.138	0.048	0.008	0.013	0	0	0.041	0.093	0.042	0.200	0.075	0.605

APPENDIX A
Continued

Locus*	Allele†	Population														
		United States Texas	Mexico Monterrey	Mexico Tapachula	Guatemala Pacific	Guatemala Atlantic	Belize	Grenada	Colombia	Ecuador	Peru	Chile	Argentina	*An. franciscanus*	*An. crucians*	*An. albimanus*
Hk-1	n	54	79	174	80	209	131	77	21	95	151	114	87	10	40	43
	152	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.000
	146	0	0	0	0	0	0	0	0	0	0	0	0	0	1.000	0
	117	0	0.006	0.009	0	0.002	0.004	0	0	0	0	0	0	0	0	0
	100	1.000	0.994	0.977	0.988	0.988	0.702	1.000	1.000	1.000	1.000	0.996	1.000	1.000	0	0
	86	0	0	0.014	0.013	0.010	0.294	0	0	0	0	0.004	0	0	0	0
	(H)	0	0.013	0.046	0.025	0.024	0.305	0	0	0	0	0.009	0	0	0	0
Hk-2	n	54	79	174	80	209	133	77	21	95	151	114	80	9	40	43
	195	0	0	0	0	0	0	0	0	0	0	0	0	0	0.013	1.000
	166	0	0	0	0	0	0	0	0	0	0	0	0	0	0.975	0
	131	0	0.006	0.011	0	0.002	0.004	0	0	0	0	0.018	0	0.778	0.013	0
	100	1.000	0.994	0.986	0.994	0.988	0.944	1.000	0.976	0.989	1.000	0.982	0.975	0.222	0	0
	71	0	0	0.003	0.006	0.010	0.053	0	0.024	0.011	0	0	0.025	0	0	0
	(H)	0	0.013	0.029	0.013	0.024	0.098	0	0.048	0.021	0	0.035	0.050	0.222	0.050	0
Idh-1	n	54	10	12	8	121	4	16	21	31	20	32	13	10	4	3
	116	0.037	0	0.042	0.063	0.062	0.375	0	0.024	0.016	0	0	0	0.050	0	1.000
	100	0.963	1.000	0.958	0.938	0.938	0.500	1.000	0.976	0.984	1.000	1.000	0.962	0.950	0	0
	83	0	0	0	0	0	0.125	0	0	0	0	0	0.038	0	0.250	0
	71	0	0	0	0	0	0	0	0	0	0	0	0	0	0.625	0
	52	0	0	0	0	0	0	0	0	0	0	0	0	0	0.125	0
	(H)	0.074	0	0.083	0.125	0.124	1.000	0	0.048	0.032	0	0	0.077	0.100	0.750	0
Idh-2	n	54	10	12	8	120	3	16	21	31	20	32	13	10	4	3
	133	0	0	0	0	0	0	0	0	0	0	0	0.038	0	0	0
	100	1.000	1.000	1.000	1.000	0.988	1.000	1.000	0.976	0.871	1.000	1.000	0.962	0	0	0
	59	0	0	0	0	0.013	0	0	0.024	0.129	0	0	0	1.000	0	1.000
	(H)	0	0	0	0	0.025	0	0	0.048	0.194	0	0	0.077	0	0	0
Lap-1	n	54	90	108	66	195	115	79	18	95	145	108	95	10	35	40
	108	0	0.006	0.037	0.008	0.015	0	0	0	0.005	0.003	0.023	0.037	0	0	0.025
	100	0.963	0.828	0.958	0.909	0.959	0.996	0.994	1.000	0.984	0.983	0.977	0.947	1.000	0.157	0.050
	94	0.037	0.167	0.005	0.083	0.026	0.004	0.006	0	0.011	0.014	0	0.016	0	0.843	0.200
	89	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.675
	82	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.050
	(H)	0.074	0.233	0.083	0.152	0.082	0.009	0.013	0	0.032	0.034	0.046	0.084	0	0.314	0.375
Lap-2	n	48	70	150	56	118	77	80	18	64	69	52	49	4	24	43
	106	0	0	0	0	0	0	0	0	0	0	0	0	0	1.000	0
	100	1.000	0.993	0.953	0.964	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0	0
	92	0	0.007	0.047	0.036	0	0	0	0	0	0	0	0	0	0	0
	(H)	0	0.014	0.080	0.071	0	0	0	0	0	0	0	0	0	0	0
Ldh	n	54	90	172	80	209	127	79	15	95	142	108	90	10	40	43
	109	0	0	0	0	0	0	0	0.167	0	0	0	0.056	1.000	1.000	0
	100	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.833	1.000	0.989	1.000	0.939	0	0	0
	91	0	0	0	0	0	0	0	0	0	0.011	0	0.006	0	0	1.000
	(H)	0	0	0	0	0	0	0	0.333	0	0.021	0	0.122	0	0	0

APPENDIX A
Continued

Locus*	Allele†	Population: United States Texas	Mexico Monterrey	Mexico Tapachula	Guatemala Pacific	Guatemala Atlantic	Belize	Grenada	Colombia	Ecuador	Peru	Chile	Argentina	*An. franciscanus*	*An. crucians*	*An. albimanus*
Mdh	n	54	90	171	80	209	127	79	21	95	145	108	95	10	40	43
	−75	0	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0
	−81	0	0.006	0.003	0	0	0.008	0	0	0	0	0.014	0.005	0	0	1.000
	−100	1.000	0.989	0.997	1.000	1.000	0.992	1.000	1.000	1.000	1.000	0.986	0.995	1.000	1.000	0
	(H)	0	0.022	0.006	0	0	0.016	0	0	0	0	0.028	0.011	0	0	0
Me	n	54	89	170‡	80	209	127	79	22	95	145‡	108	92	10	40	43
	110	0	0	0.015	0	0	0.004	0	0	0	0.017	0.005	0.005	1.000	0	1.000
	100	1.000	0.994	0.976	1.000	1.000	0.996	1.000	1.000	0.989	0.983	0.995	0.995	0	0	0
	86	0	0.006	0.009	0	0	0	0	0	0.011	0	0	0	0	0	0
	81	0	0	0	0	0	0	0	0	0	0	0	0	0	1.000	0
	(H)	0	0.011	0.035	0	0	0.008	0	0	0.021	0.021	0.009	0.011	0	0	0
Mpi	n	54	80‡	176	70	207	108	70	21	95	143	114	92	10	36	43
	100	0.861	0.950	0.983	1.000	0.998	0.991	1.000	0.976	0.989	0.983	1.000	0.995	0	0	0
	91	0.139	0.038	0.006	0	0.002	0.005	0	0.024	0.011	0.017	0	0.005	1.000	0	0
	83	0	0.013	0.011	0	0	0.005	0	0	0	0	0	0	0	0.264	0
	76	0	0	0	0	0	0	0	0	0	0	0	0	0	0.736	0
	(H)	0.167	0.063	0.034	0	0.005	0.019	0	0.048	0.021	0.035	0	0.011	0	0.417	0
6Pgd	n	54	89	187	78	209	138	95	21	95	156	114	94	10	39	43
	225	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.012
	191	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.965
	147	0	0.022	0.011	0.006	0.002	0	0	0.119	0	0.013	0	0.043	0	0	0.023
	128	0.009	0	0	0.006	0	0	1.000	0	0	0.003	0	0.085	0	0	0
	100	0.991	0.944	0.984	0.981	0.990	0.989	0	0.857	0.958	0.785	1.000	0.388	1.000	0.974	0
	54	0	0.028	0.003	0.006	0	0.011	0	0.024	0.042	0.199	0	0.399	0	0.026	0
	23	0	0.006	0.003	0	0.007	0	0	0	0	0	0	0.085	0	0	0
	(H)	0.019	0.112	0.032	0.038	0.019	0.022	0	0.190	0.084	0.314	0	0.596	0	0.051	0.070
Pgi	n	54	90	172	80	209	127	79	22	95	145	114	95	10	40	43
	100	1.000	0.994	1.000	1.000	0.998	1.000	1.000	1.000	1.000	0.997	1.000	1.000	1.000	0	0
	97	0	0.006	0	0	0.002	0	0	0	0	0.003	0	0	0	1.000	1.000
	(H)	0	0.011	0	0	0.005	0	0	0	0	0.007	0	0	0	0	0
Pgm	n	54	88	187	78	209	127	95	21	95	156‡	114	96	10	40	42
	151	0	0.023	0.005	0.006	0	0	0	0	0	0	0.013	0	0	0	0.976
	123	0.120	0.102	0.099	0.058	0.036	0.854	0	0.619	0.516	0.971	0.961	0.995	0	0	0.012
	100	0.815	0.841	0.880	0.929	0.952	0.138	1.000	0.381	0.479	0.016	0.026	0.005	0.900	0.975	0.012
	75	0.065	0.034	0.016	0.006	0.012	0.008	0	0	0.005	0.013	0	0	0.100	0.025	0
	(H)	0.370	0.239	0.193	0.141	0.086	0.236	0	0.381	0.463	0.045	0.079	0.010	0.200	0.050	0.048
Pk-1	n	54	89	166	80	205	112	65	21	95	145	108	95	10	40	43
	120	0	0	0	0.006	0.007	0	0	0.024	0	0	0	0	0	1.000	1.000
	117	0	0.017	0.003	0.006	0	0	0	0	0.016	0	0	0	0	0	0
	100	1.000	0.983	0.997	0.988	0.993	1.000	1.000	0.976	0.984	1.000	1.000	1.000	1.000	0	0
	(H)	0	0.034	0.006	0.025	0.015	0	0	0.048	0.032	0	0	0	0	0	0
Pk-2	n	54	89	172	80	205	133	71	21	95	145	113	96	10	40	43
	126	0	0	0	0	0	0	0	0.024	0	0	0	0	0	1.000	1.000
	100	1.000	1.000	1.000	0.994	1.000	0.966	1.000	0.976	1.000	0.997	0.881	1.000	1.000	0	0
	78	0	0	0	0.006	0	0.034	0	0	0	0.003	0.119	0	0	0	0
	(H)	0	0	0	0.013	0	0.053	0	0.048	0	0.007	0.239	0	0	0	0

APPENDIX A
Continued

Locus*	Allele†	Population														
		United States Texas	Mexico Monterrey	Mexico Tapachula	Guatemala Pacific	Guatemala Atlantic	Belize	Grenada	Colombia	Ecuador	Peru	Chile	Argentina	*An. franciscanus*	*An. crucians*	*An. albimanus*
Sdh	n	52	90	169	80	209	127	79	22	92	145	108	95	9	32	43
	119	0.077	0.011	0.012	0.006	0.033	0.008	0	0	0	0	0	0.026	0.056	0	0
	100	0.875	0.967	0.979	0.988	0.950	0.992	1.000	1.000	0.652	0.959	1.000	0.905	0.944	0	0
	80	0.048	0.022	0.006	0.006	0.017	0	0	0	0.348	0.041	0	0.068	0	0	0
	69	0	0	0.003	0	0	0	0	0	0	0	0	0	0	0	0
	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0.953	0
	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0.047	0
	(H)	0.250	0.067	0.041	0.025	0.100	0.016	0	0	0.478	0.083	0	0.179	0.111	0.094	0
Tpi-1	n	54	73	148	72	193	109	80	22	95	137	108	88	10	40	29
	111	0.083	0	0	0	0	0	0	0	0	0	0	0	0	0	1.000
	100	0.917	1.000	0.986	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.994	0.500	0	0
	88	0	0	0.007	0	0	0	0	0	0	0	0	0.006	0	0	0
	75	0	0	0.007	0	0	0	0	0	0	0	0	0	0.500	1.000	0
	(H)	0.167	0	0.027	0	0	0	0	0	0	0	0	0.011	0.400	0	0
Tpi-2	n	54	89	172	80	209	127	80	22	95	145	108	96	10	39	43
	138	0.056	0.006	0.003	0.006	0.005	0	0	0	0	0	0	0	0	0.026	0.977
	100	0.944	0.994	0.988	0.994	0.990	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.962	0.012
	60	0	0	0.009	0	0.005	0	0	0	0	0	0	0	0	0.013	0.012
	(H)	0.074	0.011	0.023	0.013	0.019	0	0	0	0	0	0	0	0	0.077	0.047

* For definitions of loci, see Table 2.
† n = no. of specimens; H = heterozygosity (direct count) per locus; Negative values indicate cathodally migrating alleles.
‡ Locus deviating from Hardy-Weinberg equilibrium.